ON THE ROUTE OF ABSORPTION OF BACTERIA FROM THE PERITONEAL CAVITY.*

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IT has been a generally accepted teaching that the chief route of absorption of the contents of the great serous cavities is furnished by the lymphatic system, and although the possibility has been sometimes admitted that soluble substances might to some extent diffuse into the blood vessels of the serosa, yet it seems to have been assumed that all formed particles must escape through the lymph channels. In recent years it has been shown by Asher, Starling, Mendel,[1] and others that absorption and removal of soluble substances introduced into the peritoneal cavity is accomplished through the blood vessels to a large extent, indeed, apparently to even a greater extent than through the lymphatics. In performing experiments which involved consideration of the route of absorption of a finely divided emulsion from the peritoneal cavity,[2] it was found that the emulsion did not appear in the lymph flowing from the thoracic duct in any appreciable quantities, and the question then presented itself: Is the assumption which is current among pathologists and surgeons, that bacteria leave the peritoneal cavity in peritonitis chiefly or solely by way of the lymph stream, a correct assumption? Are there any proofs that this is the route followed by the bacteria in entering the blood from the peritoneal cavity. Is it not probable that bacteria migrate directly into the blood vessels fully as readily as into the lymphatics?

Many *a priori* arguments in favor of the latter view presented themselves. For example, our views of absorption of formed particles by way of the lymphatics rather than by the blood stream are largely the outcome of the long-cherished belief that the lymphatic capillaries communicate directly with the serous cavities by means of stomata, and hence offer an open avenue of escape for bacteria and similar insoluble particles; yet there now seem to be ample grounds

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[1] *Amer. Jour. Physiol.*, 1899, 2, p. 342.

[2] WELLS AND MENDEL, *Amer. Jour. Physiol.*, 1907, 18, p. 156.

for believing that the lymphatics are closed vessels, and if so they should be no more readily entered by bacteria than the blood capillaries. Another argument, also, is the accumulating evidence that bacteria injected into the peritoneum appear in the systemic blood stream in large numbers within a very few minutes, a rapidity quite at variance with the known slowness of the lymph flow. Furthermore the lymph glands should offer a decided barrier to rapid passage of bacteria into the blood, if they exercise the function commonly ascribed to them.

The literature contains several reports upon the question of the absorption of bacteria from the peritoneal cavity, but in none of them has it been definitely determined to what extent bacteria pass from the peritoneum to the blood by way of the lymphatic system. To be sure, several observations have been made bearing on this point in one way or another, but the positive proof obtainable by studying the bacterial content of the lymph flowing from the thoracic duct seems not to have been sought; at least no reference to such studies has been found in any of the more recent literature on the subject of peritoneal absorption.

The most important observations on the absorption of bacteria from the peritoneal cavity have been made by Buxton.[1] This investigator found that if large numbers of typhoid bacilli (one-half an agar slant) are injected into the peritoneal cavity of rabbits, large numbers of bacilli appear in the blood very quickly. The largest numbers are found in blood taken from five to fifteen minutes after the inoculation, although this large number generally persists in the blood for thirty minutes or so, and then decreases rapidly, so that as a rule after one hour few bacteria can be found in the blood. This "rush" of bacteria into the blood is considered by Buxton to occur by way of the lymphatics, which the bacteria enter through the diaphragm. In support of this view is advanced the fact that at a correspondingly early period after inoculation large quantities of bacteria may be found in the anterior mediastinal glands, into which converge the lymphatic vessels of the diaphragm on their way to the thoracic duct. It has been found that small foreign particles other than bacteria, such as granular pigments, follow the same route,

[1] *Jour. Med. Res.*, 1906, 15, pp. 1–89; 1907, 16, pp. 17–42, 251.

Muscatello having found that in dogs carmine particles injected into the peritoneum reach these lymph nodes in five to seven minutes. According to Buxton's observations there is little if any absorption of pigment granules by any part of the peritoneal surfaces besides the diaphragm and omentum, but in both these structures the absorption of particles is extremely rapid, and they soon appear in the anterior mediastinal glands. No more positive evidence than the above seems to have been obtained that the route of entry of bacteria into the blood is through the lymphatics, and there seems to have been no attempt made to determine whether bacteria do or do not pass from the peritoneum into the blood stream directly.

In view of the accumulating evidence that soluble substances, both in the peritoneum and elsewhere, are absorbed directly into the blood, it is pertinent to inquire if bacteria may not do likewise. There are a number of observations recorded by Buxton that might be interpreted as evidence in favor of the direct absorption of bacteria into the blood. First, the fact that the blood contains the largest number of bacteria a very few minutes after intraperitoneal inoculation is more readily understood if bacteria can pass directly into the blood vessels than if they must, before reaching the blood, be first absorbed into the diaphragmatic lymphatics, from there pass to the mediastinal nodes, be filtered through the tortuous sinuses of one or more of these, and from there pass through another set of lymphatic vessels to the thoracic duct, and thus reach the blood. Anyone who has observed the very slow dripping of the thoracic lymph from a cannula in the duct of even a large animal, will be inclined to question the probability that any considerable number of bacteria can make this journey and swarm into the blood in great numbers in the space of five minutes. Again, the large numbers of bacteria found in the lymph nodes might be reasonably interpreted, not as bacteria on their way to the blood, but rather as bacteria kept back from the blood by the lymph nodes, which are, supposedly, interposed as a barrier to prevent just such a rush of bacteria into the blood. In such a set of experiments by Buxton, in which relatively small numbers of bacteria (20,000 to 50,000), were injected, it was found that a considerable number can be recovered from the blood, anterior mediastinal lymph glands, and liver when these are removed ten to fifteen

minutes later. "Organs other than the liver contain so few bacteria after these minimal doses that they have been left out of account in making up the table."[1] Might this observation not be interpreted in favor of the view that bacteria not only enter the lymph stream from the peritoneum, but also enter the portal vessels directly? If the bacteria entered the blood through the thoracic duct only, we should expect them to be at least as abundant in the other viscera as in the liver, which receives but little blood that has not already been filtered through two separate sets of capillaries. But if bacteria in the peritoneum can pass into both the blood and lymph directly, we should expect to find them arrested chiefly in the first two filters of these vessels, the liver and the anterior mediastinal glands, and this is exactly what Buxton has found to be the case. In Buxton's tables in the same article[2] it will be obserevd that there are about as many bacteria recovered from the anterior mediastinal glands as are present in the entire liver or in the entire blood of the animal. This might be interpreted as indicating either that absorption from the peritoneum by the lymphatics is about as great as by the blood, assuming that the lymph glands and the liver are equally effective as filters; or, in view of the very great bactericidal power of the blood as shown in these and other experiments, it is quite possible that even more bacteria are absorbed by the blood than by the lymph, since only part of the bacteria absorbed into the blood remain in a viable condition so that they can produce colonies when inoculated into plates.

In a later article[3] it is stated that at first there is a greater deposition of bacteria in the liver than in the spleen, but after four to six hours the number in the spleen increases until this organ contains more than the liver, and their eventual disappearance from the spleen is slower than from the liver. Is it not possible to interpret this observation as indicating that at first bacteria reach the blood chiefly by direct absorption into the portal capillaries, so that they are first found most abundantly in the liver; later, when they have slowly made their way through the lymphatics to the thoracic duct, they enter the general circulation, and the spleen exercises its highly developed phagocytic function to remove them from the blood?

[1] BUXTON, *Jour. Med. Res.*, 1907, 16, p. 25.

[2] *Loc. cit.*, pp. 28–31.

[3] *Jour. Med. Res.*, 1907, 16, p. 254.

As can be seen, we find in the results of Buxton's careful experiments no conclusive evidence in favor of the prevailing assumption that bacteria are absorbed from the peritoneal cavity either chiefly or exclusively by the way of the lymphatics; but rather, find excellent reasons for believing that bacteria may enter the blood vessels directly as well as the lymph vessels, exactly as has been found in studies of the absorption of soluble substances.

The only definite consideration of the pros and cons of the question of the direct absorption of bacteria into the blood that we have found, is contained in the recent article by W. Noetzel.[1] This author quotes the observations of Schimmelbusch, that bacteria may be absorbed from fresh wounds directly into the blood, where they may be found in ten minutes. Noetzel found that anthrax bacilli injected into the peritoneal cavity of animals appear in the circulating blood in ten minutes. *Bacillus pyocyaneus* injected into the pleura was found in the blood in an equally brief space of time. He discusses the question of the possibility of the rate of the lymphatic flow being great enough to account for so rapid a transportation of bacteria from the serous cavities into the blood, and performed experiments that seem to answer it affirmatively. These experiments were as follows: Bacteria (*B. pyocyaneus*) were injected into the knee joints of rabbits care being taken to avoid any injury to blood vessels, apparently with the idea in mind that bacteria can enter the blood vessels only when these have been injured. The bacteria were found in the blood stream as early as five minutes after such injection, and Noetzel believes them to have been transported solely by the lymphatics, since he had injured no blood vessels. Of more importance is his observation that the inguinal, crural, and lumbar lymph glands, removed five or ten minutes after injection of the bacteria into the knee joint, contained the same variety of organism; the numbers were not ascertained. This seems to be good evidence that absorption of bacteria from the synovial sacs takes place in a very few minutes, and that bacteria may pass in this time beyond the first sets of interposed lymph nodes. Dismissing the possibility that bacteria can enter the uninjured blood vessels of the synovial sacs directly, without having disproved it, Noetzel considers that all the bacteria

[1] *Beitr. z. klin. Chir.*, 1906, 51, p. 740.

found in the blood must have passed through the lymphatic system from the knee joint to the thoracic duct; to account for this he introduces evidence that the lymph stream may escape passing through the lymph nodes, because of the presence of anastomosing branches of the lymph vessels in the capsule of the lymph nodes which connect the afferent and efferent lymph vessels, and also through branches connecting the larger trunks directly with one another. As a result of his studies and deductions he is very sceptical as to the value of the lymph nodes as filters of the lymph, and doubts that they have the protective function against bacterial invasion so universally ascribed to them.

Other writers on the subject of the absorption of bacteria seem entirely to have neglected consideration of the route by which the absorption takes place, or else they have assumed that the lymphatics offer the only possible route. Apparently they have all been influenced by the current view that the lymphatics open directly into the serous cavities, and hence offer a free avenue of escape to the bacteria, while believing, as does Noetzel, that the only formed substances that can possibly pass through the walls of the blood capillaries are the leucocytes. If the lymph capillaries do not have stomata opening directly into the serous cavities, as seems to have been established definitely by histological and embryological studies, then there is no evident reason why bacteria may not enter the blood capillaries exactly as readily as they enter the lymph capillaries. However, it is not to be denied that there are many features of absorption, both of solid particles and of dissolved substances, that are much more difficult to explain if there are no stomata than if the presence of stomata is assumed, and at the present time it would seem unwarranted to refuse to admit the possibility that there may be functional stomata, even although they are not anatomically demonstrable.

With the above facts and hypotheses in mind, we have attempted to ascertain by direct experiment: First, whether bacteria injected into the peritoneal cavity do or do not find their way into the thoracic duct, and if so, after what length of time they reach the subclavian vein. Second, whether they enter the blood without passing through the thoracic duct. The experiments have given us very conclusive

results as far as they have been carried out, and therefore it seems desirable to publish them although there are many features of the problem still left undecided, since we are unable to continue the work at this time.

The experiments were performed as follows: Dogs were anesthetized with A.C.E. mixture, after a preliminary dose of morphine. A sterile paraffined cannula was inserted into the femoral artery and another into the main trunk of the thoracic duct just before it enters into the vein, the fluid from the latter dripping into a sterile graduated cylinder. An emulsion containing the washings from several agar slants covered with a 24-hour growth of bacteria in 0.85 per cent salt solution was injected by means of a blunt pipette into the peritoneal cavity through an incised opening, and the peritoneal wound closed by either a purse-string suture or by artery forceps in order to bring peritoneal surfaces in contact. Blood was drawn off at intervals from the cannula in the femoral artery into sterile test tubes, always first allowing enough blood to escape to ensure getting blood from the general circulation; 0.5 c.c. was then measured off in a sterile pipette, mixed with melted agar and plated out before coagulation occurred. We had no difficulty in securing cultures in this way without having the blood clot, and did not find it necessary to use bile or other coagulation-inhibiting substances. At similar intervals two drops of the lymph were allowed to fall into a tube of melted agar, and plated out. When streptococci were being used in the experiments the lymph was drawn into freshly made blood agar, in order to increase the growth and to facilitate the recognition and counting of the colonies. With rabbits the procedure was the same, except that we were unable to insert a cannula into the thoracic duct, therefore the duct when found was divided, and when not found all the tissues where the duct lies, and also the great veins in the vicinity, were securely occluded by forceps to shut off the lymph from the blood. Hence in the rabbits we did not make cultures of the thoracic lymph itself, but determined how many bacteria were present in the blood of animals with this channel shut off, and how many were in the blood of normal rabbits of the same size treated in the same way except that the thoracic duct was untouched.

The results of the experiments were as follows:

Experiment II.[1]—A large male hound, weighing about 25 kilos, was treated as above described. One hundred and twenty-five c.c. of salt solution containing the growth on 10 slants of agar during 24 hours, were injected into the peritoneal cavity. From the cannula inserted in the thoracic duct lymph flowed at the rate of 6 c.c. in the first 10 minutes, and 3 c.c. in 10 minutes in the middle of the experiment, which is about the normal rate for dogs of this size. The cannula was not paraffined in this experiment, and became occluded frequently by clots, which had to be removed with a sterile platinum wire; therefore there occured considerable contamination of some of the plates, but in the figures given below all but the colon colonies are disregarded.

TABLE 1.

COLON COLONIES FROM LYMPH FROM THORACIC DUCT (2 DROPS USED FOR EACH PLATE).

Time after Injection	Number of Colonies
2 min.	0
7 "	0
14 "	0
21 "	0
32 "	87
45 "	210
53 "	132
66 "	500
89 "	10
108 "	41
118 "	18

Cultures made from the femoral blood during the same period failed in every case to show colonies of colon bacilli.

This experiment furnishes evidence that colon bacilli when, injected in great numbers into the peritoneum of dogs, enter the thoracic duct and begin to reach the thoracic duct blood in about half an hour; after the end of the first hour the number rapidly decreases. In the same animal no living bacilli were present in the blood drawn from the femoral artery. A similar result was obtained in the following experiment:

Experiment III.—Male dog, weighing about 10 kilos, treated in the usual way. Besides the main thoracic duct, two small branches were found entering the vein; these were ligated and a paraffined cannula inserted into the main trunk. The lymph flow was always good, the rate being 10 c.c. in the first five minutes, and from 5 to 7 c.c. in 10 minutes during the middle of the experiment. One hundred cubic centimeters of salt solution containing 24 hours' growth of colon bacilli on seven agar slants were injected into the peritoneal cavity, and the results of the culures were as follows:

[1] The first experiment was of doubtful value because of faulty technique, the cannula in the thoracic duct becoming frequently occluded by clots, so that no lymph could be obtained. Cultures from the blood made during two hours did not show any colon colonies.

TABLE 2.

Time after Injection	Colonies from Lymph (2 Drops)
5 min.	0
12 "	10
18 "	53
25 "	81
32 "	279
41 "	311
48 "	188
57 "	180
69 "	45
81 "	14
99 "	10
111 "	6
119 "	3

In none of the cultures from the blood of the femoral artery were colon colonies found.

Here again we find a large number of colon bacilli reaching the end of the thoracic duct in a half-hour, the flow being somewhat more rapid than in the previous experiment in which the cannula became frequently occluded by clots, with the number decreasing rapidly in an hour. Likewise no colon bacilli could be found in the arterial blood during the two hours the experiment was continued. These results would seem to indicate that bacteria escaping from the peritoneal cavity of dogs reach the blood only through the thoracic duct, and do not pass directly into the blood. To control this, however, it was necessary to ascertain the number of bacteria reaching the femoral blood of dogs with intact lymphatic vessels, as in the following experiment:

Experiment IV.—Male dog, weighing about 7 kilos, anesthetized in the usual way, and injected intraperitoneally with 100 c.c. of salt solution containing 10 slants of colon culture. The neck region was left untouched, and plate cultures of the blood from the femoral artery were made at frequent intervals during two hours. *In not a single plate did colon colonies appear.*

Although the thoracic duct of this dog was intact, and living bacteria were passing into the blood in the lymph at a rate of several hundreds or thousands a minute, if we can judge by the results of other experiments, yet no bacteria could be found in the blood escaping from the femoral artery. Evidently the power of the blood of the dog to kill colon bacilli as used in this experiment is great enough to destroy all those reaching it from the peritoneum, either through the lymph or directly, and consequently our first two experiments prove only that bacteria pass from the peritoneum into the lymphatics; they do not prove that bacteria are not absorbed directly

into the blood from the peritoneal cavity. In the hope that we might be able to learn something about this possibility a similar set of experiments was performed with a strain of virulent streptococci, which it was thought might be able to survive the bactericidal power of the dog's blood long enough to be detected in the arterial blood. These experiments resulted as follows:

Experiment V.—Brindled female, very fat, weighing about 15 kilos, was prepared in the usual way. The thoracic duct was found and ligated, but having difficulty because of the very great obesity of the animal, did not succeed in inserting a cannula. Injected into the peritoneal cavity 100 c.c. of salt solution containing streptococci from five blood-agar slants of streptococci, grown 24 hours.

TABLE 3.

Time after Injection	Colonies from Blood (0.5 c.c.)
2 min.	0
5 "	0
15 "	0
45 "	0
60 "	3
75 "	0
90 "	4
105 "	1

Experiment X.—Small male dog, weight 5 kilos, anesthetized as usual. Cannula inserted in femoral artery; nothing done to thoracic duct. Injected 100 c.c. of salt solution containing five slants of streptococci grown 48 hours.

Made cultures at intervals for an hour from the blood, but obtained *no* streptococcus colonies.

Experiment XIII.—Large male dog, weighing about 20 kilos. Cannula inserted in thoracic duct, and obtained a good flow of lymph; 7 c.c. in the first ten minutes, and the same rate at the end of one hour. Injected into the peritoneum 100 c.c. of salt solution containing 10 slants of streptococcus, 48 hours old. The results of the cultures were as follows:

TABLE 4.

Time after Injection	Colonies from Lymph (2 Drops)	Colonies from Blood (0.5 c.c.)
2 min.	0	..
7 "	0	0
15 "	2	7
20 "	15	..
25 "	....	0
30 "	100*	..
35 "	1,000	0
40 "	2,000	..
45 "	50	0
60 "	30	1
75 "	40	0
90 "	...	0
105 "	...	0
120 "	...	0

* Numbers approximated in this column.

These experiments show that streptococci, like colon bacilli, reach the thoracic duct in considerable numbers after about half an

hour, but the number present in the lymph soon decreases about as rapidly as it begins. We also find evidence that streptococci may possibly get from the peritoneum into the blood by some other route than the thoracic duct, since a few bacteria were found in the blood in Experiments V and XIII, although it is possible that they may simply have passed from some anastomosing branch of the thoracic duct to the lymphatics entering the veins on the right side of the neck. As the control animal, No. X, with the thoracic duct intact, showed no cocci in the blood from the femoral artery, it is impossible to tell whether the small number of cocci found in the blood of the other two animals indicates that the number of bacteria reaching the blood by some route other than the thoracic duct is small, since it is evident that such bacteria would soon be destroyed through the bactericidal power of the blood. It seems that the latter factor is so great in dogs that they are unsuitable for determining this point, and hence experiments were performed with rabbits. In rabbits the thoracic duct is so small and delicate that we were unable to insert a cannula, but contented ourselves with occluding it and comparing the bacterial content of the blood of such animals with those in which the thoracic duct was untouched. Apparently the bactericidal power of the blood of rabbits for colon bacilli is not so great as in dogs, for there was no difficulty in finding great numbers of bacteria in the blood soon after they had been injected into the peritoneum, as shown by the following experiments:

Experiments VI and VII.—Two rabbits, three-fourths grown, of about equal size. Into the peritoneal cavity of each were injected 25 c.c. of salt solution containing one-half the growth of colon bacilli on an agar slant of 24 hours. With No. VI, cultures were made at intervals from the femoral artery. With No. VII, the thoracic duct was first isolated and cut, so that no lymph reached the blood from this source. The results of the blood cultures in each case were as given below:

TABLE 5.

Time after Injection	VI (Control) Colonies from blood (0.5 c.c.)	VII (Duct Cut). Colonies from Blood (0.5 c.c.)
1 min.	0	0
3 "	1	0
5 "	272	0
10 "	66	0
15 "	75	12
20 "	21	2
25 "	22	0
30 "	5	0
35 "	2	0
40 "	?	0
50 "	6	0
60 "	3	0

Experiments VIII and IX.—In these experiments the conditions were the same as in the previous pair, except that, being unable to find the thoracic duct, it was occluded by artery forceps grasping the tissues where it lies, while other forceps shut off from the general circulation that part of the jugular and cervical veins into which the lymph could possibly be discharged. The results were as follows:

TABLE 6.

Time after Infection	IX (Control). Colonies from Blood (0.5 c.c.)	VIII (Duct Occluded). Colonies from Blood (0.5 c.c.)
5 min.	0	0
10 "	0	0
15 "	560	0
25 "	1,270	0
35 "	460	0
45 "	590	0

TABLE 7.

EXPERIMENTS XI AND XII. CONDITIONS THE SAME AS ABOVE.

Time after Injection	XI (Control). Colonies from Blood (0.5 c.c.)	XII (Duct Occluded). Colonies from Blood (0.5 c.c.)
5 min.	240*	44
10 "	240	66
15 "	350	..
25 "	Spoiled	Lost
35 "	2,000	87
50 "	3,000	21
60 "	2,000	0

* Numbers approximated in this column.

These experiments seem to show conclusively that when colon bacilli are injected in large numbers into the peritoneal cavity of rabbits, they pass into the blood chiefly by way of the thoracic duct, for when the duct is cut or occluded relatively few or no bacteria can be found in the arterial blood. The bacteria reach the blood in a very few minutes, and either decrease rapidly after the first "rush" of bacteria, or else they may continue to increase (Exp. XI), presumably because of deficient defensive powers of the blood. Since in two of these three experiments a greater or less number of colon bacilli were found in the blood in spite of the occlusion of the thoracic duct, it seems probable that they can enter the blood by some other route. It must be emphasized, however, that as in experiments such as these in which the thoracic duct is occluded a stasis of the lymph current is produced, so that the conditions of absorption are not normal. It is quite possible that such occlusion of the main duct causes the lymph to reach the blood by collateral branches

passing to the lymph duct on the right side, or possibly by some other less understood route. Therefore the finding of bacteria in the blood of rabbits with occluded thoracic ducts cannot be considered positive proof that the bacteria have entered the blood directly rather than by way of the lymphatic vessels. It would be very interesting to study the bacterial content of the portal blood of animals receiving intraperitoneal injections of bacteria, but we have been unable to devise a method of doing this in the living animal without inflicting severe peritoneal trauma and making the conditions of absorption altogether abnormal.

SUMMARY.

Evidence has been secured that when colon bacilli or streptococci are injected in large numbers into the peritoneal cavity of dogs or rabbits, they begin to enter the blood through the thoracic duct in considerable numbers after from 15 to 30 minutes in the dog, and from 5 to 15 minutes in the rabbit. The difference in the length of time probably depends upon the distance the organisms have to travel in the slowly moving lymph. It is possible that some bacteria enter the blood by some other route than the thoracic duct, but whether the route consists of collateral lymph channels, or direct passage into the peritoneal blood capillaries, we have not ascertained; neither have we been able to determine positively whether bacteria do or do not escape directly from the peritoneum into the blood. There is a striking rush of bacteria into the thoracic lymph, so that the maximum number is found almost at once after the first bacteria appear, and begins to decrease in half an hour or less; this corresponds with the observations of Buxton upon the appearance of bacteria in the circulating blood after their injection into the peritoneum.

It would seem probable that the mechanism of absorption of bacteria from the peritoneal cavity is quite different from the mechanism of absorption of soluble substances, since the latter seem to pass directly into the blood even more rapidly and abundantly than into the lymph. This is comparable to the condition of absorption in the intestine, the soluble sugar entering the blood directly by diffusion while the emulsionized fat escapes through the lymphatics.